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ADHESIVE INTERACTIONS BETWEEN THE TUBE FEET OF A STARFISH, *LEPTASTERIAS HEXACTIS,* AND SUBSTRATA

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ABSTRACT

The tube feet of *Leptasterias hexactis* adhere to and release from substrata by chemical interactions. In our laboratory these podia adhered to substrata coated with the ubiquetous anionic saccharide films produced by marine bacteria. Podia also attached to moderately anionic glass, but not to uncharged surfaces. The adhesive epithelia of tube feet labeled heavily with ruthenium red, indicating they were anionic. Tube feet secreted footprint films that bound crystal violet, a cationic dye. Trypsin removed the films. Adhesion to marine surfaces was prevented by 300 units/ml of heparin, a glycosaminoglycan (GAG) that may have competitively inhibited the glue from binding exosaccharide marine films. Lectins that bind bacterial exosaccharides did not inhibit attachment. We propose that tube-foot attachments are nonspecific ionic interactions established by secreted proteinaceous films and released when secreted GAG's compete with the tube-foot epithelium for sites on the film. This system agrees with the duo-gland model for adhesion and deadhesion.

INTRODUCTION

The tube feet of echinoderms, and asteroids in particular, are traditionally viewed as miniature suction cups that are aided in their attachment to substrata by adhesive secretions (Smith, 1947; Thomas and Hermans, in press). The functional morphology of the tube feet of *Asterias rubens* was analyzed by Smith (1947) who developed a model that describes how tube feet may function as suction cups. This model ignored chemical ("mucus") adhesion although the work of Paine (1926) was cited. Paine (1926) had concluded that roughly 44% of the adhesive forces in the tube feet of *A. vulgaris* comes from chemical adhesion. The question of how each tube foot is chemically attached and detached during the pedal locomotory cycle has been raised and needs to be resolved (Hermans, 1983).

During preliminary observations for this study a *Leptasterias hexactis* in our laboratory adhered to a somewhat corroded, well-used stainless steel plankton screen (10 mesh/mm). Although it was not entirely clean, the mesh was open, and the starfish adhered so well that tube feet broke off and remained on the net when the animal was pulled free. The average diameter of the adhesive discs of *L. hexactis* is 1.0 mm. It would have been impossible for the podia to have used suction for attachment to the screen.

Anyone observing starfish in aquaria will note that the tube feet generally remain clean during the attachment/detachment/reattachment cycle in locomotion. Adherent material does not accumulate on the adhesive surfaces. If there is a chemical adhesive it is either torn away from the tube foot at each step or a chemical mechanism frees each podium of adherent material. Kerkut (1953) demonstrated that tube feet ordinarily

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are not torn from the substratum at each step during normal locomotion. He showed that the tube feet do not stick to or pull upon the substratum as they are lifted away. Electron micrographic studies have described cells that were claimed to secrete adhesives (Harrison and Philpott, 1966; Chaet, 1965), but Hermans (1983) suggested that the putative adhesive granules may be deadhesive or cleansing in function and that actual adhesive materials may be produced by other cells.

We agree with Roth (1983) that biological adhesion mediated by proteins binding carbohydrates may have evolved only once, and, observing that biological adhesive relationships are often temporary, we believe that duo-gland relationships, in which adhesiveness is modulated by secretions that promote adhesion or flow, are wide spread but not recognized in many cases (Hermans, 1983). The study of echinoderm tube feet will shed light on duo-gland, adhesion/flow or adhesion/detachment relationships generally. Therefore we have examined some chemical interactions between the tube feet of *Leptasterias* and various substrata under varied conditions.

Materials and Methods

The starfish used in this study were members of the genus *Leptasterias,* small six-rayed forcipulates, collected from the mid-intertidal zone at Shell Beach, Sonoma County, California. They were maintained in marine aquaria at 12°C. The current taxonomy of *Leptasterias* is unclear (Fisher, 1930; Chia, 1966; Sutton, 1975). The animals used in this study were collected from a single population on one large boulder, and a few nearby rocks. The specimens ranged from 2 to 5 cm diameter and conformed most closely to Fisher's (1930) description of the polymorphic species *Leptasterias hexactis* (Stimpson). These starfish were chosen because of their availability, convenient size, and because they are similar to a variety of the most common species of starfish found in temperate waters.

Tube feet were collected by pulling *Leptasterias* from rocks. The tube feet that tore from the sea star by remaining attached to the rock were fixed in glutaraldehyde-ruthenium red, and postfixed in osmium tetroxide-ruthenium red, according to Mellonig (1976). Some starfish were allowed to attach to *Phyllospadix,* a plant normally found in the habitat of *Leptasterias,* and the tube feet were severed and fixed while still attached to the plant. These were examined microscopically using the methods of Thomas and Hermans (in press).

Footprints left by walking starfish were obtained by allowing starfish to walk across clean glass slides (Scientific Products). These were stained with 0.005% aqueous crystal violet and examined with a Leitz compound microscope equipped with an Ortholux camera.

The adhesiveness of tube feet to various substrata was determined in the following manner. Substrata were placed in the bottoms of finger bowls (4½ inch diameter glass culture dishes from Carolina Biological Supply, Burlington, North Carolina 27215) and covered to a depth of about 0.75 cm (50 ml) of fresh seawater or other medium. The finger bowls had been cleaned with 1% acetic acid in 95% alcohol and rinsed in seawater before using. Starfish were added and allowed to attach to the substrata in the dishes. When an animal began to move in a particular direction, its forward progress was impeded by gentle, finger tip pressure against the advancing edge. Starfish that had attached to the substratum offered finger-tip resistance that was clearly tangible. Unattached starfish float free at the slightest touch. Only animals that adhered normally to surfaces in the aquaria before and after each experimental test were counted. Starfish were placed repeatedly on the experimental surfaces because the starfish exhibited

considerable "free will." By "free will" we mean that attachment to surfaces does not appear to be governed by one simple reflex. Starfish often fail to adhere to substrata upon which they have previously demonstrated the ability to attach. For instance, excessive handling or attempting to harness a starfish to a tensiometer inhibits attachment behavior. Stroking the backs (aboral surfaces) of the animals with light finger pressure, however, was found to stimulate indifferent animals to increase adhesion on suitable substrata. It was not possible to stimulate any adhesion on certain substrata (see Results).

The surfaces tested were teflon tape, Parafilm, dental wax, polystyrene culture dishes, 4½" diameter glass culture dishes (Carolina Biological Supply, soda lime glass), rubber, glass microscope slides, Medcast epoxy resin (Ted Pella, Co.), and clean glass microscope slides coated with sebum from otherwise clean human skin.

The distribution of negatively charged sites on substrata was visible by staining with 0.005% aqueous crystal violet for one minute and rinsing with deionized water. Crystal violet binds quantitatively to negatively charged sites (Maroudas, 1975). A violet color imparted to the surface gave a clear visual impression of negatively charged sites on surfaces, and the relative intensity of the color indicated the relative numbers of negative sites per unit area.

The increase in the relative numbers of negatively charged sites per unit area on the surfaces of glass culture dishes maintained in fresh aerated seawater for periods up to several months was measured in the following way. The culture dishes were drained, and the inside bottom of each dish was stained with 20 ml of 0.005% aqueous crystal violet for two minutes and rinsed with 50 ml of deionized water. The crystal violet that had adhered to the surface was eluted in 20 ml of 95% ethanol with gentle agitation for two minutes. The absorbance of the alcoholic crystal violet elutant was measured at 593 nm with a Bausch & Lomb Spectronic 2000 spectrophotometer. The absorbance of each sample is proportional to the number of negatively charged sites per unit area of culture dish bottom.

The effects of the divalent cations Mg and Ca on the adhesion of tube feet to surfaces were investigated using solutions of 0.01 *M* and 0.005 *M* EGTA or EDTA (disodium form) in seawater, pH 7.4, with the osmolarity adjusted to that of Northern California seawater (950 mOsm). Normal seawater contains 0.04 parts calcium ion per 100 parts seawater (0.01 *M*) and slightly less than twice that amount of magnesium ion, both relative to the chlorinity of the seawater (Sverdrup *et al.*, 1942). Each mole of EGTA binds two moles of calcium and each mole of EDTA binds one mole of divalent cations nonspecifically. The surfaces tested were clean glass slides that bore inherent negative surface charges (determined by crystal violet staining) and natural marine films formed on the clean culture dishes that had been soaked in fresh, aerated seawater at 12°C for six weeks. Five starfish were placed on one of the experimental surfaces in a culture dish containing normal seawater and allowed to attach. The seawater was poured off and enough EDTA/seawater solution was added to cover all the tube feet. The animals were gently dislodged from their attachment and given one minute to re-attach. The solution was poured off and replaced with fresh seawater, and the animals were given another one minute to re-attach.

The relationship between adhesion and the layer of calcium cations that accumulate near negatively charged substrata in solution (Adamson, 1982; Fletcher *et al.*, 1980) was studied by allowing culture dishes to equilibrate for 20 minutes with 50 ml of 0.2 *M* EGTA in normal seawater. The chelating solution was poured off and the bowl was rapidly rinsed and refilled with 50 ml of normal seawater. Starfish were quickly added and their ability to adhere was recorded. The control for this test consisted of

allowing the starfish to attach to the test dish before the substratum was chelated. This established the ability of the sample animals to attach firmly to the unchelated marine film.

The influence of heparin on the binding of tube feet to clean anionic glass slides was determined. These tests were conducted at Shell Beach and all solutions were kept at seawater temperature. Filtered natural seawater was used throughout. Fresh starfish were used for each test (n = 5). Thirty milliliters of filtered seawater were added to culture dishes containing glass slides (Scientific Products). This was enough seawater to cover the tube feet, but not the aboral disc of the starfish. Starfish were allowed one minute to adhere; only adherent starfish were used for the tests. The seawater was then replaced with 30 ml of heparin/seawater solution. After five minutes any still-adherent starfish were gently dislodged and allowed ten minutes to re-attach. The heparin solution was then replaced with natural seawater and the starfish were allowed ten minutes to re-attach. The test solutions consisted of natural filtered seawater and heparin sodium salt (Sigma Chemical Company) at concentrations of 300, 150, and 75 units/ml. At these concentrations the heparin had no effect on the pH and added not more than 0.4 mOsm to the seawater.

The possibility that lectins play a role in the adhesion of tube feet to naturally occurring marine films was investigated using films that developed on culture dishes as described above and 0.05 *M* solutions of those sugars, in seawater, that are known to be major components of gram-negative bacterial films. Some of these sugars have been shown to inhibit lectin binding in other systems (Kirchman *et al.*, 1982, 1984; Sutherland, 1972, 1980; Brown *et al.*, 1969 and Williams *et al.*, 1979). The monosaccharides tested are listed in Table I.

To gain some information on the chemical composition of the glue produced by tube feet a study was made of the effects of various chelating agents (EDTA and EGTA) and enzymes (trypsin, chymotrypsin, alpha amylase, and beta amylase) on footprints left on glass slides by starfish that had adhered to glass slides and were then gently removed. The slides with the footprints on them were immersed in Coplin jars of either test or buffer solutions at 22°C for one hour, and then stained with either crystal violet or aqueous carmine. The following test solutions were used: aqueous EDTA (0.2 *M*), saturated aqueous EGTA, 0.5 mg/ml trypsin or chymotrypsin in 0.2 *M* phosphate buffer (pH 8.0), alpha amylase in pH 5.5 phosphate buffer, and beta amylase in pH 3.5 acetate buffer. The enzymes were purchased from Boehringer Mannheim Biochemicals.

RESULTS

The surfaces of the *Phyllospadix*, to which *Lepatasterias* had adhered when fixed, was covered by a bacterial film 1 μm thick containing rod-shaped and spherical gram negative bacteria 300 nm in diameter embedded in a fibrillar matrix (Fig. 1). Between

TABLE I

Monosaccharides

αL(-)-Fucose	D(+)-Glucosamine
D(+)-Galactose	3-0-α-methyl glucose
2-Deoxy-D-Galactose	D-Mannose
D-Galactosamine	α-Methyl-D-mannose
D-Glucose	D-Ribose
1-Deoxy-D-Glucose	

the bacterial layer and the adhesive surface of the tube feet there was a descrete layer of amorphous substance that averaged 2.2 μm in thickness.

The adhesive surfaces of the tube feet bound ruthenium red strongly, forming an electron-dense coat that obscured the glycocalyx and microvilli (Fig. 2).

When starfish walked across clean glass microscope slides they left footprints that stained heavily with crystal violet (Fig. 3). When the staining and rinsing were done gently these footprints retained thick films of material that stained in a reticular pattern. These films were the same diameter as the adhesive epithelium on each tube foot and corresponded to the deposits seen beneath attached podia in electron micrographs. The reticular pattern in each film consisted of heavily stained boundaries that separated larger areas that stained more lightly than the boundaries, but more heavily than the surrounding glass slide. The clean glass microscope slides to which tube feet adhered stained evenly and lightly with crystal violet.

The tube feet did not adhere equally well to all surfaces. There was a positive correlation between adhesivity, moderate negativity (crystal violet binding) and hydrophilic (water-drop tests) surfaces. These results are summarized in Table II.

Significant increases in the density of negatively charged sites developed as clean glass was soaked in fresh seawater (Fig. 4).

Starfish attached to previously uncharged and non-adherent glass surfaces after they acquired natural marine films. But if the films became too thick, they became slimy, and starfish could no longer adhere. This occurred when one batch of glass was kept for several months in a marine aquarium that had been inoculated with bacteria on rocks from a quiet region of Bodega Bay. The dishes became heavily coated with a negatively charged slime of bacteria, diatoms, algae, and other small organisms. These conditions could not have developed on the outer coast where *Leptasterias* is found, because wave action and grazing animals would have reduced the film before it approached the thick slimy non-adherent stage. When these heavily coated glass surfaces were superficially cleaned with a strong stream of water or gentle wiping, so that they were still coated but no longer slimy, the starfish adhered well.

Starfish adhered firmly in culture dishes that had developed marine thin films in normal seawater before and after immersion of the tube feet in 0.01 *M* or 0.005 *M* solutions of EDTA in seawater, but did not adhere to the same surfaces while in this solution. Podia that had attached before ordinary seawater was replaced by seawater containing EDTA did not spontaneously detach, yet once their hold on the bottom of the glass dishes had been broken they did not reattach in the presence of EDTA. The podia moved normally in the chelating agent for only about a minute. If left in the solution for longer than three minutes they gradually became noticeably less active.

The results of the same tests using 0.01 *M* and 0.05 *M* solutions of EGTA in seawater were consistant with those for EDTA. Starfish that were attached did not detach spontaneously when the EGTA was added; after being dislodged they did not re-attach until after the EGTA solution was replaced with normal seawater. The tube feet retained normal levels of activity during the time course of these experiments, but if left in the chelating agent for long periods they became inactive. Nevertheless, they still adhered well in normal seawater before and after being in the solution of chelating agent.

Tube feet failed to adhere to clean glass slides in 0.01 *M* EGTA, but they did adhere lightly in 0.005 *M* EGTA and were normally adherent in the control tests with ordinary seawater before and after the experimental solutions. Under control conditions the tube feet left footprints on the glass slides. They left some footprints when they were in 0.005 *M* EGTA solution. They did not deposit footprints on the glass in solutions of 0.01 *M* EGTA.

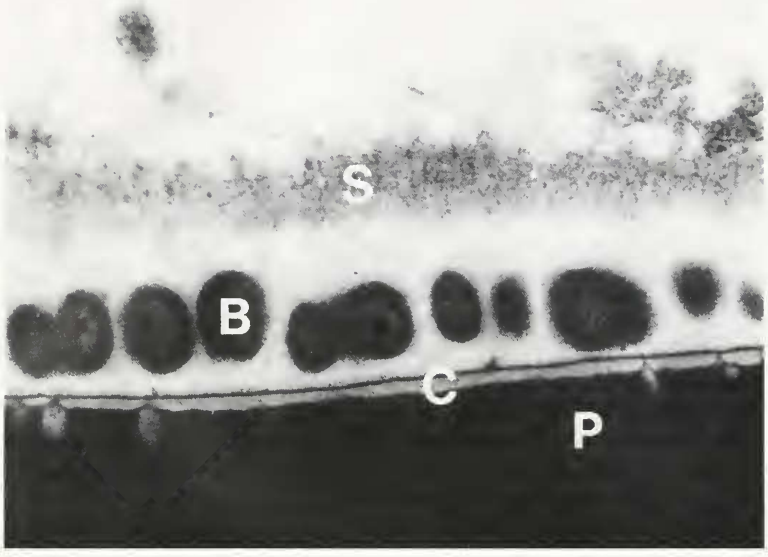

FIGURE 1. Gram-negative bacteria coating the surface of *Phyllospadix*. B = bacterium; C = cuticle of *Phyllospadix;* P = *Phyllospadix;* S = tannic-acid labeled bacterial exosaccharide film. 30,000×.

Starfish in normal seawater adhered poorly to substrata coated with marine films that were chelated with 0.2 *M* EGTA for 20 minutes immediately before starfish were placed upon them.

FIGURE 2. Electron micrograph of section through adhesive surface of tube-foot epithelium showing the apical glycocalyx (G) heavily labeled with ruthenium red. 10,000×.

FIGURE 3. Light micrograph of adherent film (footprint) left on glass slide by walking starfish and stained with crystal violet. 40×.

Footprints that were soaked in chelating solutions for one hour appeared entirely normal when stained with crystal violet and examined microscopically. Neither EDTA nor EGTA disrupted the footprint films.

Heparin at a concentration of 300 units/ml prevented starfish attachment. It also caused the release of previously attached animals within five minutes. At 150 units/ml only three out of five animals spontaneously released, and detached animals could form weak bonds to the substratum within five minutes. After five minutes in a solution of 75 units/ml all animals remained attached, and within five minutes after

TABLE II

Ability of tube feet to bind various substrata compared to the anionic charges (crystal-violet binding) and hydrophobicity (water-drop tests) of those surfaces

Substratum	Tube-foot adhesion	Crystal-violet stain	Water-drop test
Glass slides	Firm	Yes	Hydrophilic
Rubber	Firm	Yes	Hydrophilic
Marine film on glass dish	Firm	Yes	Hydrophilic
Clean glass dish	None	No	Hydrophobic
Teflon tape	Weak	Uneven	Uneven
Polystyrene	Weak	Uneven	Uneven
Parafin	None	No	Hydrophobic
Dental wax	None	No	Hydrophobic
Epoxy resin	None	No	Hydrophobic
Sebum	None	Yes	Intermediate

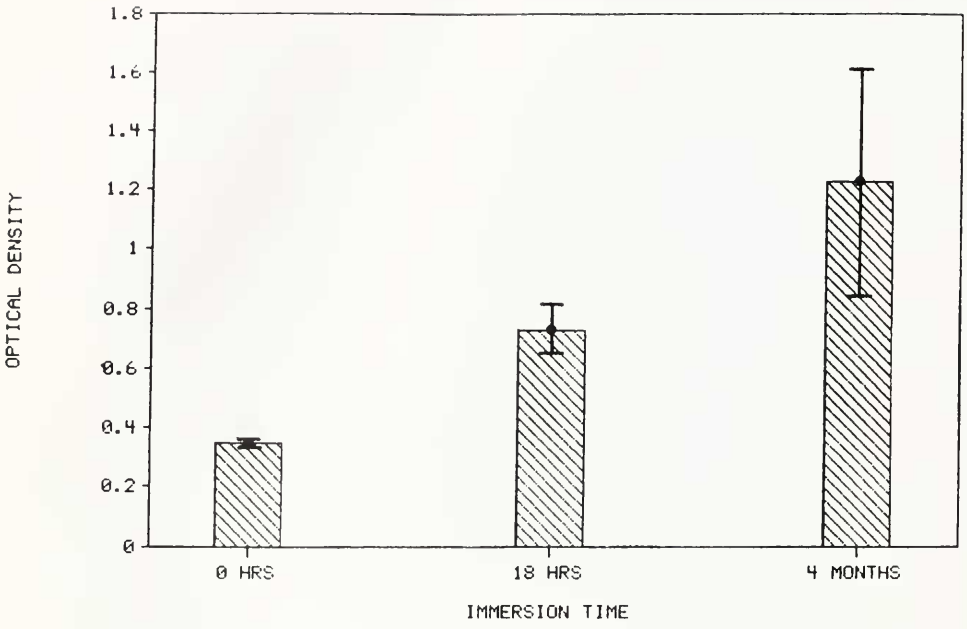

FIGURE 4. Relative amounts of crystal violet staining insides of bottoms of glass culture dishes per unit surface area. Average absorbance of 0.354 (absorbance units at a wave length of 593 nm, S.D. = 0.009) on recently cleaned glass; average absorbance of 0.731 (S.D. = 0.08 on glass that had been soaked in aerated fresh seawater for 18 hours); average absorbance of 1.2288 (S.D. = 0.386) on glass that had been soaked in aerated fresh seawater for four months.

being dislodged there was some re-attachment. All starfish were able to re-attach firmly within five minutes after replacement of the heparin solutions with normal filtered seawater.

Trypsin removed all traces of footprints from glass slides. Examination under both the dissecting microscope and the Leitz compound microscope revealed clean slides, devoid of all matter, and crystal violet did not bind footprint sites. Control prints soaked in the same buffer as the trypsin solutions were completely intact and stained well.

Prints treated with amylases were disrupted but remained on the slides, and tube feet which were severed from the starfish and remained attached to the slides were still present after the amylase digestions. Footprints treated with beta amylase appeared less disrupted than those treated with alpha amylase.

None of the monosaccharides used as potential competitive inhibitors of lectins prevented tube feet from adhering.

DISCUSSION

The epithelium on the adhesive surface of the tube feet of *Leptasterias* contain three types of cells: adhesive cells, large-granule secreting cells, and monociliated cells. The adhesive cells contain small dense granules and have broad distal surfaces covered with microvilli that bear numerous fine filaments at their tips. These cells also contain large bundles of intermediate filaments that connect the adhesive surface of each cell with the tension-bearing network of collagen between and beneath the cellular elements

of the adhesive epithelium. Cells that secrete large granules are arranged alternately with the adhesive cells. The large granules are released through narrow collars of microvilli that form secretory channels between the glycocalyces of the neighboring adhesive cells and deliver the granules between those glycocalyces and the substrata to which they adhere. The monociliated cells scattered throughout the adhesive epithelium are probably sensory (Thomas and Hermans, in press).

In this study we have demonstrated that the glycocalyx covering the adhesive surface of the tube foot stains heavily with ruthenium red. This fact indicates that the glycocalyx bears many negatively charged sites (Luft, 1971, 1976). Thus the entire distal surface of the tube foot is coated by a negatively charged surface which somehow attaches to substrata.

Tube feet, like cells *in vitro,* do not attach equally well to all substrata. In both cases the cells attach to charged substrata that have sufficient rigidity to support the tensions exerted on them (Maroudas, 1973, 1975). *Leptasterias* tube feet do not attach to uncharged surfaces (Parafilm, dental wax, epoxy resin, glass slides from Scientific Products) or to charged surfaces that are lacking in rigidity (sebum, very thick marine films). The water-drop test, which measures the contact angle of a drop of water placed on a substratum, is generally used as an index of hydrophobicity, but according to Maroudas (1973, 1975) this test is not sufficiently sensitive to detect small charged sites that are nevertheless adequately large to serve as points of attachment on otherwise hydrophobic surfaces. Maroudas (1973) found that small impurities provide enough charged sites for the attachment of cells to otherwise hydrophobic surfaces *in vitro.* The moderately anionic surfaces to which tube feet adhere may seem hydrophobic by water-drop standards when compared with highly charged surfaces, but their true characteristics are apparent when hydrophobic materials such as paraffin are included in the tests. This explains why tube feet were moderately adhesive with respect to polystyrene and teflon in spite of these surfaces' general hydrophobicity. They contain sufficient anionic sites, as revealed by crystal violet, to make adhesion possible. We also observed that glass surfaces, usually considered to have similar characteristics, sometimes differ significantly in surface charge. Some glass samples (cleaned culture dishes, Carolina Biological) frequently lacked sufficient charged sites for podia to attach (crystal violet test), but glass slides (Scientific Products) did have sufficient charge, and tube feet attach well on them. However, highly charged negative surfaces are repellent to tube feet, and may even be toxic (Sechler and Gunderson, 1974).

To understand the mechanisms by which starfish attach and detach, we must understand the characteristics of the moderately negative microbial films that coat all marine surfaces (Characklis, 1981; Neihof and Loeb, 1974). Brewer (1984) described such films as hydrophobic, but our own tests confirm that they are negatively charged and that bacteria can double (from 0.354 to 0.731 absorbance units, see results) the number of negatively charged sites on glass in less than a day. The films consist of macromolecules and bacteria that are deposited in a two-step process. When immersed in seawater, materials with differing wettabilities, surface tensions, and electrophoretic mobilities are instantaneously coated with an organic molecular layer, usually polysaccharides and glycoproteins (Characklis, 1981). Electropositive or strongly electronegative surfaces are unlikely to exist in natural seawater because they rapidly adsorb this dissolved organic material, and it imparts a characteristically moderate negative charge to the surfaces (Neihof and Loeb, 1974). The adsorbed organic layer conditions the surface with nutrients and provides a slightly negative surface charge. It then attracts motile marine bacteria, and they, as well as randomly arriving non-motile bacteria, attach to the molecular film (Marshall, 1974).

Irreversible bacterial attachment occurs when bacteria produce polysaccharide

polymers that are acidic due to numerous uronic acid groups. These polysaccharide fibers utilize calcium, magnesium, and iron for intermolecular bonding that creates the polyanionic carbohydrate slime characteristic of marine surfaces (Characklis, 1981; Corpe, 1970, 1974). Many studies support the theory that bacterial films form the preferred substratum for the attachment of other marine organisms (Meadows and Williams, 1963; Bracato *et al.,* 1982; Kirchman *et al.,* 1982, 1984; Brewer, 1984).

In the case of *Phyllospadix* we have demonstrated that the substratum to which tube feet attach is a bacterial film coating the surface of the plant (Fig. 1). Since the glycocalyx on each podium and the bacterial film coating *Phyllospadix* are acidic in nature, the chemical adhesive material must be capable of cross-linking acidic surfaces. The numerous microvilli projecting from the adhesive cells provide structural reinforcement to the glue matrix as it coats the highly irregular surfaces found in marine environments.

Chemical attachment of the adhesive epithelium to a substratum occurs over the entire contact surface of the disc. Thomas and Hermans (in press) showed that the adhesive epithelium is morphologically uniform, and the evenly labeled footprints left by firmly attached starfish indicate that the epithelium is also functionally uniform. These complete footprints did not contain unstained regions such as those described by Smith (1947) for *Asterias.* However, the animals frequently used little attachment when crossing level surfaces in glass dishes. Under these circumstances the podia apparently serve as levers and struts (Kerkut, 1953) that propel the animal forward while expending little glue for actual attachment. The ability to regulate the release of glue is energetically advantageous and explains the high variability of adhesiveness observed in individual tube feet of the same size (Paine, 1926). It also provides for the fact that tube feet that manipulate eggs and larvae (Chia, 1968) can also adhere to substrata with a force that exceeds the tensile strength of the stems.

The footprints deposited by tube feet on glass slides are films with negatively charged surfaces, and they correspond in size and pattern to the adhesive epithelium of the podia. The broad areas in the footprints that stain lightly with crystal violet correspond to the large surfaces of the adhesive cells, and the narrow more darkly stained bands correspond to the pattern formed by the cells that secrete the large granules between the adhesive cells (Fig. 3).

The fact that footprint films stain with crystal violet suggests that at least one of the components is acidic. One explanation for this acidity is that the secreted glue might consist of glycosaminoglycans (GAG's) that use divalent cations to link acidic glycocalyces to acidic substrata. This model has been proposed for bacterial adhesion (Fletcher *et al.,* 1980). However, the footprint films left by walking tube feet were not disrupted by either the general divalent cation chelating agent EDTA or the calcium-chelating agent, EGTA. Assuming that EGTA had access to any calcium ions, and that electrostatic repulsion between the GAG's and EGTA did not inhibit the chelating activity, it appears that divalent cations are not essential for the integrity or adhesion of the footprint films. However, calcium ions are clearly important in the adhesive process of podia, especially the calcium-ion concentration that probably accumulates at negatively charged surfaces (see discussion of double electrical layers in Adamson, 1982). Tube feet in EGTA-chelated water initially retained their normal mobility, but could not attach to the substrata, and they adhered poorly even in normal seawater when the substrata had been chelated with EGTA. We conclude from this that calcium is not an essential element in the structure or function of podial glue, but that it is required by the exocytotic process of secretion. Since EGTA is quite specific for calcium ions, it is apparent that neither magnesium nor other divalent cations, which would

have remained in the test solutions of EGTA, can substitute for calcium in the adhesive process.

There is an alternative model to explain the anionic content of footprint films. The footprints may be composed of a basic protein released from the small dense granules in the adhesive cells and coated with negatively charged polymers released from the cells secreting the large granules from the adhesive epithelium. Accordingly, basic proteins may directly link the negatively charged glycocalyces on the adhesive epithelium to negatively charged substrata, or more likely, either basic or amphoteric proteins could polymerize into filaments linking the tube feet to the substrata. The thick matrix seen in micrographs of the interface between attached tube feet and *Phyllospadix* support the polymer hypothesis (Thomas and Hermans, in press). With basic proteins forming the glue, detachment of tube feet could be accomplished by the release of glycosaminoglycans (GAG's) from the large granules. The GAG's would compete with the glycocalyx for sites on the glue, thus releasing the tube foot and leaving a footprint film consisting of glue coated with GAG's on the substratum. This releasing factor may be similar to the GAG heparin, which releases podial attachments at higher concentrations and prevents attachment at lower concentrations. The anatomy and distribution of large-granule producing cells is appropriate for the delivery of such a product to the interface between glycocalyx and glue.

This basic protein/GAG model for adhesion and release is supported by the fact that the most strongly negative parts of the footprint films correspond to the apices of the large-granule secreting cells and the fact that the comparable large granules in other species of starfish have been demonstrated to contain GAG's (acid mucopolysaccharides) (Harrison and Philpott, 1966; de Sousa Santos and Sasso, 1968). The fact that thorium stained only the outer matrix of the large granules (Harrison and Philpott, 1966) can be explained by the fact that thorium penetrates very poorly.

The fact that the carbohydrate-digesting enzymes, alpha amylase and beta amylase, failed to completely release footprint material from glass slides, whereas digestion with trypsin and chymotrypsin did, supports this model. Although the glue may contain complex carbohydrates not digested by amylase it is still probable that the protein moiety binds the films to glass. Whereas, most of the saccharides in footprint films may be from the releasing agent, a small amount may be associated with the adhesive protein, if it is similar to known extracellular adhesive glycoproteins such as fibronectin (Yamada, 1983a, b).

The inhibition of podial attachment by heparin lends further support to this model. Heparin is a highly acidic polysaccharide that binds to the charged amino residues of basic proteins. According to our model heparin may prevent podial attachment by binding to cationic sites on the proteinaceous glue thereby preventing the glue from attaching to the acidic groups of the substrate film.

Tube feet do not attach with lectins that bind the monosaccharides common to bacterial films. We might have expected lectins to play a role because some marine invertebrates do use lectins when attaching to substrata (Kirchman *et al.*, 1982). In the latter case, however, the larvae of sessile organisms are apparently being aided in selecting appropriate substrata for permanent attachment by the specificity of the lectins involved. A mobile starfish like *Leptasterias* would not be aided by such a specific attachment mechanism.

Suction has been regarded as the primary means of tube-foot adhesion. Paine (1926) concluded that 44% of podial attachment is contributed by glue and that the rest is from suction. This conclusion was based on several assumptions that are not valid with respect to *Leptasterias*. She assumed that podia adhere equally well to

various substrata. Total adhesion was tested by allowing podia to attach to glass and the adhesiveness of glue was measured by allowing them to attach to the open ends of rubber tubes. We know nothing about the characteristics of either of these surfaces. Second, it was assumed that only the periphery of a sucker uses glue for adhesion, since this adhesion was measured by allowing podia feet to attach to the open ends of tubing. The hollow center of tubing not only prevented suction from forming, but also eliminated an unknown proportion of chemical attachment. Finally, Paine assumed that the tension exerted on an attached podium at the time of release is the maximum tension sustainable. This ignores the "free will" aspect of podial release. This "free will" made it very difficult for us to collect podia attached to pieces of *Phyllospadix* because more often than not, when the plant was gently pulled upon the attached podia released. Clearly, when a small portion of substratum shifts, the podia attached to it frequently release. This response explains the large range of results Paine (1926) reported. Furthermore, when well-attached starfish are pulled from substrata the stems of many tube feet break, leaving the discs and portions of stems attached to the substrata. Obviously the ability of a disc to adhere exceeds the tensile strength of the stem. Therefore, when Paine measured the tension sustained by podia at the time of release she could not possibly have obtained the maximum tension sustainable by the adhesive epithelium. From this we conclude that Paine (1926) and others have grossly underestimated the contribution of glue to podial attachment and have failed to see the need for a chemical detachment mechanism.

Although the tube feet of starfish may also use suction on solid surfaces, it appears to be a secondary adjunct to the adhesion established by glue. The attachment of podia fits the extended model of the "contact hypothesis" of cellular adhesion presented by Maroudas (1975). According to this theory secreted polymers form bridges between cell membranes and substrate surfaces, making close molecular contact with both. In the "bridging" model the two most important characteristics of substrata are rigidity and surface charge. Because podia attach consistently to rigid, moderately negative surfaces we suggest that they attach primarily by polypeptide bridges anchored by lysine and/or arginine residues to GAG's of the adhesive-cell glycocalyces and GAG's of the bacterial films that coat marine substrata. Release is probably affected by the secretion of GAG's (perhaps similar to heparin) that compete with the anionic sites on the glycocalyx for the basic residues on the glue, thus the tube feet release cleanly leaving footprint films behind. The proteinaceous glue is probably contained in the small granules of the adhesive cells and the competitive GAG releasing factor is probably in the large granules of the narrow-necked cells. This model explains the data from Kerkut (1953) that show tube feet release cleanly from attached substrata and are not mechanically pulled free. It also accounts for the observation by Smith (1937) that when the ophuroid *Ophiocomina* is disturbed while adhering to the sides of aquaria it releases the bonds of all tube feet simultaneously and drops to the bottom of the tank. This model for adhesion of starfish tube feet accords with the model for podial adhesion suggested by Hermans (1983).

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